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Antidepressants in a changing ocean: Venlafaxine uptake and elimination in juvenile fish (*Argyrosomus regius*) exposed to warming and acidification conditions

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HIGHLIGHTS

- VFX can be uptaken by fish through both water and diet.
- Both warming and acidification decreased VFX uptake in liver.
- VFX uptake in brain was favoured under warming conditions.
- VFX elimination in liver was impaired by warming and acidification.

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ABSTRACT

The presence of antidepressants, such as venlafaxine (VFX), in marine ecosystems is increasing, thus, potentially posing ecological and human health risks. The inherent mechanisms of VFX uptake and elimination still require further understanding, particularly accounting for the impact of climate change-related stressors, such as warming and acidification. Hence, the present work aimed to investigate, for the first time, the effects of increased seawater temperature ($\Delta T°C = +5$ °C) and pCO_2 levels (ΔpCO_2 ~1000 μatm, equivalent to $\Delta pH = -0.4$ units) on the uptake and elimination of VFX in biological tissues (muscle, liver, brain) and plasma of juvenile meagre (*Argyrosomus regius*) exposed to VFX through two different exposure pathways (via water, i.e. [VFX] ~20 $\mu g\,L^{-1}$, and via feed, i.e. [VFX] ~160 $\mu g\,kg^{-1}$ dry weight, dw). Overall, results showed that VFX can be uptaken by fish through both water and diet. Fish liver exhibited the highest VFX concentration ($126.7 \pm 86.5\ \mu g\,kg^{-1}$ and $6786.4 \pm 1176.7\ \mu g\,kg^{-1}$ via feed and water exposures, respectively), as well as the highest tissue:plasma concentration ratio, followed in this order by brain and muscle, regardless of exposure route. Both warming and acidification decreased VFX uptake in liver, although VFX uptake in brain was favoured under warming conditions. Conversely, VFX elimination in liver was impaired by both stressors, particularly when acting simultaneously. The distinct patterns of VFX uptake and elimination observed in the different scenarios calls for a better understanding of the effects of exposure route and abiotic conditions on emerging contaminants' toxicokinetics.

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1. Introduction

Over the last years, pharmaceuticals and personal care products (PPCPs) have been widely detected in marine ecosystems (e.g. Arpin-Pont et al., 2016; Rodriguez-Mozaz et al., 2017). The frequent detection of PPCPs in the marine environment can be attributed to two main reasons: i) increased human usage of PPCPs along with increased world population; and ii) these chemical contaminants are not completely eliminated at conventional wastewater treatments plants (WWTPs) (Gros et al., 2012) despite the constant improvements of technology. Indeed, the cost associated to removal of contaminants in WWTPs is so high that cities municipalities cannot afford to implement such novelties, and therefore PPCPs and other chemicals are still barely removed reaching water bodies. As emerging contaminants, PPCPs lack regular environmental monitoring data and their presence in seafood species is not regulated yet. Among PPCPs, the antidepressant venlafaxine (VFX), a serotonin-norepinephrine selective reuptake inhibitor (SNRI), is one of the most commonly prescribed psychiatric drugs used to treat depression and anxiety disorders in humans, being frequently detected in the aquatic environment (Álvarez-Muñoz et al., 2015; Arpin-Pont et al., 2016; Rodriguez-Mozaz et al., 2017). Recent studies have provided compelling evidence that antidepressants can accumulate in marine biota (e.g. Álvarez-Muñoz et al., 2015), and promote adverse effects at the behavioural and physiological levels as well (e.g. Bisesi et al., 2014; Best et al., 2014; Bidel et al., 2016). Most of the studies about uptake and elimination mechanisms of these contaminants have focused on their exposure via water (e.g. Boillot et al., 2015; Huerta et al., 2016a,b; Valdés et al., 2016; Serra-Compte et al., 2018), thus not considering contaminant bioaccumulation through trophic transfer (i.e. dietary exposure; e.g. Heynen et al., 2016; Boström et al., 2017). Yet, the later also represents a major pathway of contaminant exposure, particularly in predatory fish species (Brooks, 2014). Furthermore, the potential interactive effects between these contaminants and other environmental stressors are still unclear, although they can play a key role on chemical contaminants' availability and toxicity to biota, (e.g. Noyes et al., 2009; Marques et al., 2010; Maulvault et al., 2016). Understanding how marine species will cope with the presence of multiple environmental stressors represents one of the main ecological concerns and research challenges, since climate change-related effects, such as seawater warming and acidification, are already evident in many regions of the world, posing a great threat to marine life (IPCC, 2014). Such changes are expected to worsen in the coming 50–100 years, for instance, increasing seawater temperature as high as 5 °C, as well as CO_2 partial pressures (pCO_2) up to 1000 μatm which leads to a seawater pH drop, a process known as ocean acidification (IPCC, 2014; McNeil and Sasse, 2016). Depending on the region, each stressor can occur alone or in combination with other ones, representing additional challenges to the resilience of marine ecosystems.

Seawater warming and acidification can alter contaminants' bioavailability in sediments and in water as well as the marine specie's physiological status, which may affect the way that marine organisms cope with the presence of chemical contaminants like compound uptake, retention and detoxification rates (e.g. Marques et al., 2010; Maulvault et al., 2016, 2017; Sampaio et al., 2016). Such alterations were clearly evidenced in a recent study on European seabass (*Dicentrarchus labrax*; e.g. Maulvault et al., 2016), which revealed enhanced bioaccumulation and impaired elimination of MeHg under warmer temperatures, as well as increased risks to seafood consumers. Therefore, it is of paramount importance to gather relevant data from environmental pollutants, particularly from those less well studied, such as PPCPs, enabling a better prediction of the potential implications of climate change at the ecotoxicological and public health levels.

In this context, the aim of this study was to assess, for the first time, the effects of increased seawater temperature ($\Delta T°C = +5$ °C) and pCO_2 levels (~1000 μatm, equivalent to $\Delta pH = -0.4$ units) on VFX uptake and elimination mechanisms in fish tissues (muscle, liver and brain) and plasma of juvenile meagre (*Argyrosomus regius*) exposed to the contaminant through two different exposure pathways (via water, i.e. [VFX] ~20 μg L^{-1}, and via feed, i.e. [VFX] ~160 μg kg^{-1} dry weight, dw). The selection of juvenile *A. regius* as model species was based on the following criteria: i) it is a predatory fish species, inhabiting temperate estuaries and coastal areas, thus, likely accumulating high levels of chemical contaminants (FAO, 2017); ii) environmental variations and pollution are known to be particularly deleterious to species' early life stages (including larvae and juveniles), compromising their recruitment and ecological success; and iii) it is a commercially important species and, therefore, the data obtained can be linked to seafood safety.

2. Materials and methods

2.1. Feeds (CTR and VFX-enriched) and VFX stock solutions

Two feed batches with the same nutritional composition were manufactured by the company SPAROS Lda (Olhão, Portugal): a non-contaminated feed (CTR feed) and a VFX-enriched feed. Both feeds were elaborated in order to mimic a commercial fishmeal-rich formulation for juvenile marine fish (48% crude protein and 18% crude fat; detailed feed composition is presented in Appendices, Table A.1). Briefly, all powder ingredients were grinded (<200 μm) in a micropulverizer hammer mill (Hosokawa Micron, SH1, The Netherlands), and mixed with fish oil in a paddle mixer (Mainca RM90, Spain). The feed mixture was further humidified with 25% deionized water at room temperature, and then extruded at 2.0 mm by means of a low-shear extruder (P55, Italplast, Italy). After extrusion, feed pellets were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). Then, a batch of CTR feed was subsequently enriched with a solution of venlafaxine hydrochloride ($C_{17}H_{27}NO_2 \cdot HCl$, >98%, Sigma-Aldrich) dissolved in ethanol, and further diluted with deionized water (total volume of 100 mL). A VFX nominal concentration of approximately 160 μg kg^{-1} on a dry weight basis (dw) was selected for the VFX-enriched feed (i.e. corresponding to a concentration4 times higher than the VFX levels found in species inhabiting contaminated coastal areas, susceptible to accumulate this contaminant, and that are natural preys of juvenile meagre, to assure that VFX bioaccumulation to detectable levels was elicited during the timeline of the trials; Martínez Bueno et al., 2014; Álvarez-Muñoz et al., 2015). Feed enrichment was performed by top-coating the pellets with the VFX solution using a pressurized spraying container (standard flat-fan nozzle; size 250 μm; pressure 6 bar). VFX concentration was determined in VFX-enriched feed, as well as in CTR feed to assure that no external contamination occurred during the preparation of CTR feed (Table 1).

For treatments simulating VFX exposure via water, a stock solution of VFX was prepared to perform the daily seawater spiking during the 28 days of exposure, by dissolving venlafaxine hydrochloride ($C_{17}H_{27}NO_2 \cdot HCl$, >98%, Sigma-Aldrich) with deionized water (total volume of 500 mL), in order to achieve a nominal VFX concentration of 20 μg L^{-1} in each incubating tank. Despite this value is above the concentrations usually found in seawater, the selection of VFX nominal concentration was mostly based on the order of magnitude of the lowest VFX concentration previously reported to cause significant behavioural effects in fish following short-term VFX exposure (50 μg L^{-1}; Bisesi et al., 2014). The total volume of VFX solution used to perform the daily spiking was

adjusted considering that the steady state of this compound was reached after 24 h, and accounted for the possible compound losses due to the daily 25% seawater renewal in each tank.

2.2. Fish rearing and acclimation

Argyrosomus regius specimens were reared until juvenile stage, at the aquaculture pilot station of the Portuguese Institute for the Sea and Atmosphere (EPPO-IPMA, Olhão, Portugal) using routine hatchery conditions. Subsequently, fish with similar biometric characteristics (total length: 6.8 ± 0.4 cm; total weight 2.6 ± 0.5 g) were transported to the aquaculture facilities of Laboratório Maritimo da Guia (MARE-FCUL, Cascais, Portugal), where they were randomly and equitably distributed in 21 rectangular shaped incubating glass tanks (50 L each, total volume). The incubating tanks were organized in groups of three tanks, which corresponded to one treatment (i.e. 3 replicates x 7 treatments = 21 tanks in total; Fig. 1; see the description of each treatment in Section 2.3.). Animal density was kept below 5 g body weight L^{-1} in each tank in order to avoid physiological stress related to high animal density. To maintain seawater quality, each incubating tank had independent functioning, being equipped with a protein skimmer (Reef SkimPro, TMC Iberia, Portugal), UV disinfection (Vecton 300, TMC Iberia, Portugal), biological filtration (model FSBF 1500, TMC Iberia, Portugal) and chemical filtration (activated carbon, Fernando Ribeiro Lda, Portugal; except in treatments simulating VFX exposure via water; i.e. VFX-water). Dead fish and faeces were daily removed, and seawater was partially renewed (25% of the total tank volume). Ammonia, nitrite and nitrate levels were daily checked using colorimetric tests (Tropic Marin, USA), and kept below detectable levels, with the exception of nitrates, which were kept below 2.0 mg L^{-1}. Seawater total alkalinity was also measured in each tank on a weekly basis, following the protocol previously described (Sarazin et al., 1999), and the combination of total alkalinity (AT) and pH was used to calculate carbonate system parameters (average values obtained for each treatment can be consulted in Appendices, Table A.2). Each tank had independent temperature and pH control, and daily adjustments of these parameters were performed by means of: i) temperature - an automatic seawater refrigeration system (±0.1 °C; Frimar, Fernando Ribeiro Lda, Portugal), as well as submerged digital thermostats (200 W, V2Therm, TMCIberia, Portugal); ii) pH - individual pH probes (GHL, Germany) connected to a computerized pH control system (±0.1 pH units; Profilux 3.1N, GHL, Germany), which monitored seawater pH in each tank every 2 s and adjusted whenever needed, via submerged air stones, by injecting CO_2 (Air Liquide, Portugal; to decrease pH) or CO_2-filtered aeration (to increase pH) using air pumps (Stella 200, Aqua One Pro, Aqua Pacific UK Ltd, United Kingdom).

Prior to trials, fish were acclimated to laboratory conditions for a period of 30 days, being fed with CTR feed (2% of average body weight, BW) and kept under the following abiotic conditions: i) dissolved oxygen (DO) > 5 mg L^{-1}; ii) temperature (T °C) = 19.0 ± 0.5 °C; iii) pH = 8.00 ± 0.10; iv) salinity = 35 ± 1‰; and v) photoperiod = 12 L:12D (12 h light:12 h dark). Temperature, pH, salinity and DO were daily checked using a multi-parameter measuring instrument (Multi 3420 SET G, WTW, Germany).

2.3. VFX exposure and elimination

The effect of warming and acidification acting together (i.e. the worst-case scenario) was assessed in the treatments simulating both VFX exposure routes (i.e. seawater and dietary exposures; Acid+Warm+VFX-water and Acid+Warm+VFX-feed). Yet, due to experimental limitations, only the exposure pathway through feed was selected to investigate the single effects of temperature and pH (i.e. Acid+VFX-feed and Warm+VFX-feed). The exposure via VFX-enriched feed was selected for this purpose, because: a) there is currently a lack of data on contaminant bioaccumulation through trophic transfer (i.e. dietary sources), as most of the studies focus on contaminant exposure through inhalation (i.e. water exposure); b) diet can be an important source of contaminant exposure, particularly in predatory species, often leading to more notorious toxicological effects than those promoted by contaminant exposure through inhalation (e.g. Arnot and Gobas, 2004; Brooks, 2014).

One week before initiating the trial, seawater temperature and pH were slowly adjusted (+1 °C and −0.1 unit per day), until reaching 24 °C and ~1500 µatm pCO_2 (equivalent to pH = 7.6 units) in tanks simulating climate change conditions (i.e. treatments Acid+VFX-feed, Warm+VFX-feed and Acid+Warm+VFX-feed and Acid+Warm+VFX-water; Fig. 1), according to the projections of the Intergovernmental Panel for Climate Change (scenario RPC8.5; IPCC, 2014), as well as considering the intervals of future CO_2 amplification scenarios described by McNeil and Sasse (2016).

Table 1
Venlafaxine concentration (mean ± standard deviation) in feed (µg kg^{-1} dry weight) and seawater samples (µg L^{-1}) collected from each treatment, as well as in non-contaminated fish tissues (Control treatment). Abbreviations: CTR feed: non-contaminated control feed; VFX-enriched feed: venlafaxine contaminated feed; VFX-feed – fish exposed to venlafaxine via feed; VFX-water – fish exposed to venlafaxine via seawater; Acid – simulated acidification (i.e. pCO_2 ~1500 µatm, equivalent to pH = 7.6 units); Warm – simulated warming (i.e. T = 24 °C).

Feed (µg kg^{-1}, dw)	CTR feed VFX-enriched feed	VFX concentrations < LOD 161.7 ± 17.1		
Seawater (µg L^{-1})		**Day 14**	**Day 28**	**Day 35**
	Control	< LOD	< LOD	< LOD
	VFX-feed	< LOD	< LOD	< LOD
	Acid+VFX-feed	< LOD	< LOD	< LOD
	Warm+VFX-feed	< LOD	< LOD	< LOD
	Acid+Warm+VFX-feed	< LOD	< LOD	< LOD
	VFX-water	19.7 ± 1.6	19.2 ± 1.6	16.0 ± 1.4
	Acid+Warm+VFX-water	23.2 ± 4.7	22.3 ± 2.1	15.9 ± 1.4
			Day 28	**Day 35**
Fish plasma (µg L^{-1}) and tissues (µg kg^{-1}, dw)	Control	Plasma	< LOD	< LOD
		Brain	2.6 ± 2.1	< LOD
		Liver	21.8 ± 0.6	23.0 ± 0.6
		Muscle	< LOD	< LOD

Feed: LOD = 0.30 µg kg^{-1}; Seawater: LOD = 0.15 µg L^{-1}; Plasma: LOD = 0.04 µg L^{-1}; Brain: LOD = 0.15 µg kg^{-1}; liver: LOD = 0.23 µg kg^{-1}; muscle: LOD = 0.43 µg kg^{-1}

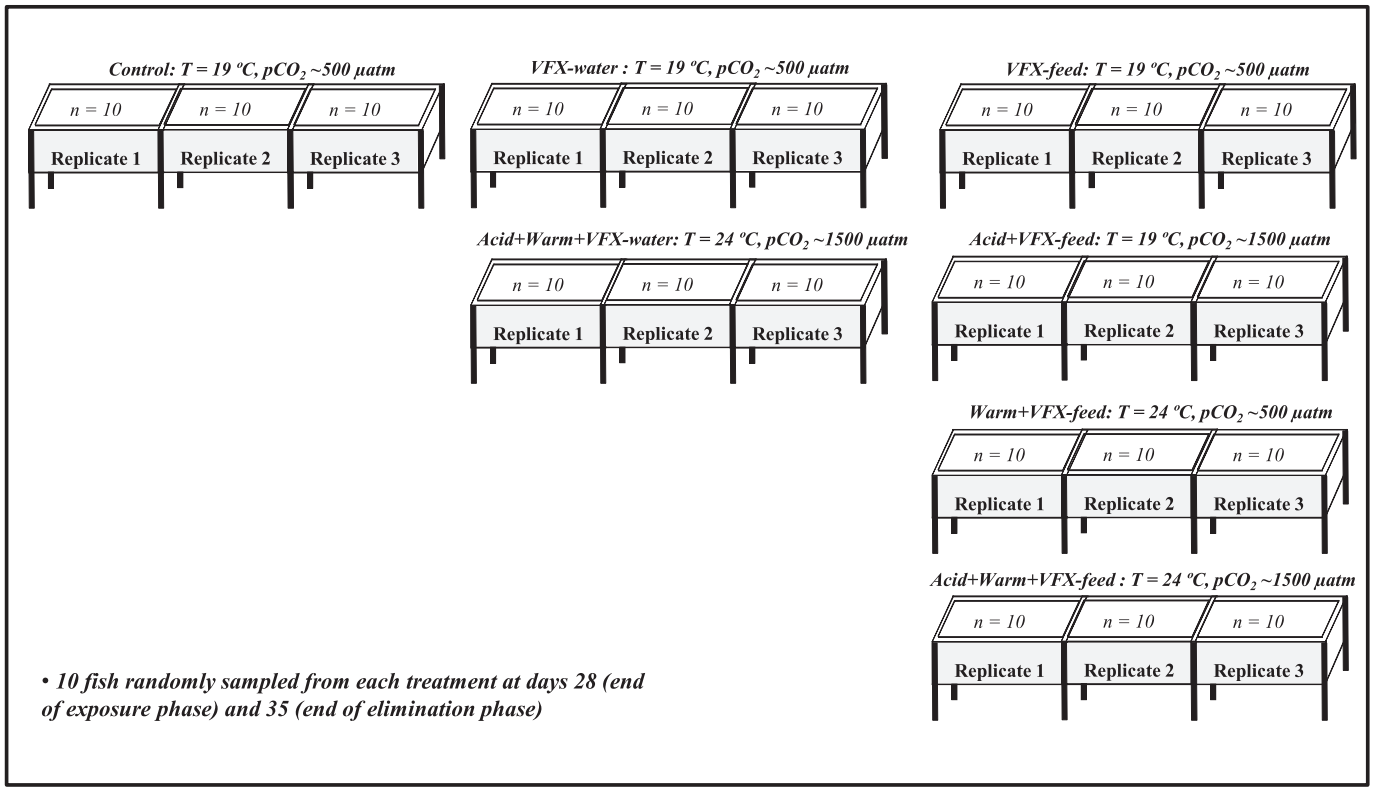
Control: T = 19 ºC, pCO2 ~500 µatm
n = 10
Replicate 1
Replicate 2
Replicate 3
VFX-water : T = 19 ºC, pCO2 ~500 µatm
VFX-feed: T = 19 ºC, pCO2 ~500 µatm
Acid+Warm+VFX-water: T = 24 ºC, pCO2 ~1500 µatm
Acid+VFX-feed: T = 19 ºC, pCO2 ~1500 µatm
Warm+VFX-feed: T = 24 ºC, pCO2 ~500 µatm
Acid+Warm+VFX-feed : T = 24 ºC, pCO2 ~1500 µatm
• 10 fish randomly sampled from each treatment at days 28 (end of exposure phase) and 35 (end of elimination phase)

Fig. 1. Experimental setup. Abbreviations: Acid – simulated acidification (i.e. pCO_2 ~1500 µatm, equivalent to pH = 7.6 units); Warm – simulated warming (i.e. T = 24 °C); VFX-water – fish exposed to venlafaxine via water; VFX-feed – fish exposed to venlafaxine via feed.

Seven treatments were carried out ($n = 10$ animals per replicate tank; Fig. 1), simulating the reference temperature (i.e. 19 °C) and pCO_2 (~500 µatm; pH = 8.0 units) conditions (i.e. temperature and pCO_2 conditions normally used in juvenile meagre rearing), as well as the projected seawater warming (ΔT°C = +5 °C) and acidification (Δ pCO_2 ~1000 µatm; equivalent to ΔpH = −0.4 units): i) Control treatment, i.e. fish daily fed with CTR feed, and simulation of the reference temperature and pH conditions; ii) VFX-water treatment, i.e. fish daily fed with CTR, seawater daily spiked with a VFX stock solution, to achieve a VFX final concentration of 20 µg L^{-1}, and simulation of the reference temperature and pH conditions; iii) Acid+Warm+VFX-water treatment, i.e. fish daily fed with CTR, seawater daily spiked with a VFX stock solution (nominal concentration = 20 µg L^{-1}), and simulation of warming and acidification (24 °C and ~1500 µatm pCO_2); iv) VFX-feed treatment, i.e. fish daily fed with VFX-enriched feed, and simulation of the reference temperature and pH conditions; v) Acid+VFX-feed treatment, i.e. fish daily fed with VFX-enriched feed, and simulation of acidification (1500 µatm pCO_2); vi) Warm+VFX-feed treatment, i.e. fish daily fed with VFX-enriched feed, and simulation of warming (24 °C); vii) Acid+Warm+VFX-feed treatment, i.e. fish daily fed with VFX-enriched feed, and simulation of warming and acidification (24 °C and ~1500 µatm pCO_2). After 28 days of exposure to VFX (day 28), a contaminant elimination phase was also carried out for a period 7 days (until day 35), i.e. fish were fed with CTR feed in all VFX-feed and VFX-water treatments, and VFX seawater spiking was stopped in VFX-water treatments (seawater also partially renewed on a daily basis during this phase, i.e. 25% of the total tank volume). During the exposure and elimination phases, fish were daily fed with the same amount (2% BW) of the corresponding feed, and feed consumption was regularly monitored throughout the entire experiment, to assure that all feed was consumed by fish from every treatment (no reduction of fish consumption was observed). Seawater abiotic parameters were daily monitored and adjusted to adequate levels whenever needed, as described above. No mortality was observed during the 35 days of the trial.

Ten fish were randomly sampled from each treatment (out of the 3 replicate tanks constituting one treatment) at days 28 and 35, and euthanized by immersion in an overdosed MS222 solution (2000 mg L^{-1}; Sigma-Aldrich, USA) buffered with sodium bicarbonate (1 g of $NaHCO_3$ and 1 g of MS222 per litre of seawater) for 10 min. Euthanized fish were measured (average total weight, TW, and total length, TL, are shown in Table 2), and blood was collected by puncture of the caudal vein and centrifuged (4 °C, 15 min, 10,000 g). Plasma samples were then collected, pooled in two composite samples (plasma of 5 individuals per pool, $n = 2$) and kept at −80 °C until further analyses. Fish were subsequently dissected to collect the muscle, liver and brain tissues, and two composite samples were also performed for each of these three tissues (i.e. muscle, liver or brain of 5 individuals per pool, $n = 2$). Composite samples were then freeze-dried at −50 °C, 10^{-1} atm of vacuum pressure for 48 h (Power Dry LL3000, Heto, Czech Republic), homogenized and stored at −80 °C until further analysis.

Seawater samples were also collected from each replicate tank (treatment) during the exposure (i.e. days 14 and 28) and elimination (i.e. day 35) phases for VFX quantification, in order to assure: i) a steady VFX concentration in tanks/treatments simulating VFX exposure via water (Table 1); and ii) no external contamination (apart from dietary exposure) was taking place in tanks/treatments simulating VFX exposure via feed (Table 1).

2.4. Venlafaxine determination

Seawater samples were filtered by PVDF syringe filters 0.22 µm (Merck Millipore) and VFX was quantified by direct injection in UPLC-QqLIT according to the methodology described by Gros et al.

Table 2
Biometric data (mean ± standard deviation) of fish collected from each treatment at days 28 and 35 of the trial. Different lower case letters indicate significant differences between Control and VFX-feed treatments, whereas upper cases letters indicate significant differences between Control and VFX-water treatments ($p < 0.05$). Abbreviations: TW – total weight; TL – total length; VFX-water – fish exposed to VFX via seawater; VFX-feed – fish exposed to VFX via feed; Acid – simulated acidification (i.e. pCO2 ~1500 μatm, equivalent to pH = 7.6 units); Warm – simulated warming (i.e. T = 24 °C).

		TW (g)	TL (cm)
Day 28	Control	$3.7 \pm 1.1^{b, B}$	$7.3 \pm 0.7^{ab, B}$
	VFX-feed	2.2 ± 0.4^{b}	6.5 ± 0.4^{b}
	VFX-feed+Acid	4.4 ± 2.8^{ab}	7.9 ± 1.4^{ab}
	VFX-feed+Warm	6.5 ± 1.2^{a}	8.9 ± 0.5^{a}
	VFX-feed+Acid+Warm	6.7 ± 2.0^{ab}	9.0 ± 1.0^{a}
	VFX-water	2.9 ± 0.7^{B}	6.8 ± 0.4^{B}
	VFX-water+Acid+Warm	7.1 ± 2.1^{A}	9.3 ± 0.9^{A}
Day 35	Control	$2.6 \pm 0.8^{b, B}$	$6.8 \pm 0.6^{c, B}$
	VFX-feed	2.4 ± 0.8^{b}	6.8 ± 0.8^{c}
	VFX-feed+Acid	2.8 ± 0.8^{b}	7.0 ± 0.6^{c}
	VFX-feed+Warm	5.0 ± 0.7^{a}	8.5 ± 0.4^{b}
	VFX-feed+Acid+Warm	7.1 ± 1.9^{a}	9.6 ± 0.6^{a}
	VFX-water	3.1 ± 1.0^{B}	7.4 ± 0.7^{B}
	VFX-water+Acid+Warm	7.6 ± 1.3^{A}	9.8 ± 0.6^{A}

(2012). VFX levels in feed (CTR and VFX-enriched) were determined following an extraction method adapted from Jakimska et al. (2013), and further quantified by UPLC-QqLIT according to Gros et al. (2012). For the determination of VFX in the fish plasma and tissues (brain, liver and muscle) at day 28 and day 35 of the experiment, the following extraction protocols were used: i) Plasma: 50 μL of plasma were mixed with 50 μL of methanol and centrifuged (5340 g, 10 min, 4 °C), 60 μL of supernatant were transferred to an insert, and 0.6 μL of a 1 ng μL^{-1} VFX-d6 standard solution was added before the analysis; ii) Brain, liver and muscle: 50 mg of freeze-dried tissue (or 25 mg in the case of brain) were extracted with 1 mL methanol:water (75:25, *v/v*) in an ultrasonic bath during 15 min. Then samples were centrifuged (8000 g, 15 min, 4 °C). This extraction procedure was repeated three times. All the supernatants were combined, evaporated until dryness under a gentle stream of nitrogen and reconstituted in 50 mL ultra-pure water with 0.1% formic acid. A solid phase extraction (SPE) using Oasis MCX (3 cc, 60 mg) (Waters) was performed for sample clean-up. Briefly, cartridges were conditioned with 3 mL methanol and 3 mL of 0.1% formic acid in ultra-pure water. Then, the reconstituted samples were percolated through the cartridges at a flow rate of 3 mL/min. The cartridges were washed with 5 mL 2% formic acid in ultra-pure water and dried under vacuum for 5 min. Finally, VFX was eluted with 6 mL 5% ammonia in methanol. The extracts were evaporated until dryness under a gentle stream of nitrogen and reconstituted in 1 mL methanol:water (50:50, *v/v*). Before analysis, extracts were filtered by PVDF syringe filters (0.22 μm) (Merck Millipore) and VFX-d6 standard solution was added as internal standard. The quantification of VFX in all samples was performed using the chromatographic method described by Gros et al. (2012). Detailed information on the validation parameters for the analysis of venlafaxine in feed, water, fish tissues and plasma is given in Appendices, Table A.3.

2.5. Data analysis

Venlafaxine net accumulation rates (NAR; μg kg^{-1} day^{-1}) for each tissue and treatment were determined assuming that fish were exposed to steady conditions (i.e. continuous contaminant exposure, as well as seawater abiotic parameters) and using the following equation (Santana et al., 2017):

$$NAR_t = \frac{[VFX_{t28}]_{tissue}}{t}$$

where, $[VFX_{t28}]_{tissue}$ is the average VFX concentration in fish tissues after 28 days of exposure. Furthermore, for VFX-water treatments, the bioconcentration factor (BCF) was also calculated after 28 days of VFX exposure using the following equation (in L kg^{-1}; Arnot and Gobas, 2016):

$$BCF = \frac{[VFX_{t28}]_{tissue}}{[VFX_{t28}]_{sewater}}$$

where, $[VFX_{t28}]_{seawater}$ is the average VFX concentration in seawater samples collected from treatments simulating VFX exposure via water, after 28 days of exposure. To facilitate comparisons with previously reported data, BCF were calculated on a wet weight (ww) basis, i.e. for brain, liver and muscle the average moisture content (79.7%, 63.1% and 79.5%, respectively) was used to convert VFX concentration values.

The percentage of VFX loss during the elimination phase (i.e. day 35), i.e. the elimination factor (EF; %) was calculated according to the following equation:

$$EF\ (\%) = 100 - \left(\frac{[VFX_{t35}]_{tissue}}{[VFX_{t28}]_{tissue}}\ x\ 100 \right)$$

where, $[VFX_{t35}]_{tissue}$ is the VFX concentration in fish tissues after 7 days of elimination (i.e. day 35; Jebali et al., 2014). EF was considered to be 0 whenever $[VFX_{t35}]_{tissue}$ was higher than $[VFX_{t28}]_{tissue}$.

In what concerns statistical analysis, the ANOVA assumptions of normality and variances homogeneity were first investigated through the Kolmogorov–Smirnov and Levene tests, respectively. Data were Log-transformed or square-rooted whenever necessary to comply with these assumptions. Then, to assess the existence of significant differences between treatments in terms of VFX tissue concentrations, three-way ANOVA analyses were performed (for both exposure routes, i.e. VFX-water and VFX-feed treatments), using tissue (brain, liver and muscle) or tissue:plasma concentration ratios, sampling day (days 28 and 35) and treatment (reference temperature and pH, warming and/or acidification) as variables. As for biometric data (total weight and total length), NAR (day 28), BCF (day 28), EF (day 35) and VFX plasma, two-way ANOVA were used instead (for both exposure routes, i.e. VFX-water and VFX-feed

treatments) to determine the existence of significant differences between treatments (biometric data: day and treatment used as variables; NAR, BCF and EF: tissue and treatment used as variables). Two-way ANOVA was also carried out to detect the presence of significant differences among treatments and sampling days in VFX plasma concentrations (results are presented in Appendices, Table A.4). Post-hoc Tukey HSD tests were subsequently carried out to identify significant differences. Finally, Pearson correlation coefficients (r) between biometric data and NAR were also calculated. Statistical analyses were performed at a significance level of 0.05, using STATISTICA™ software (Version 7.0, StatSoft Inc., USA).

3. Results

3.1. VFX levels in feed, seawater and non-contaminated fish samples

VFX concentration in feed and seawater samples, as well as in non-contaminated fish throughout the 35 days of trial is shown in Table 1. An average VFX concentration of 161.7 ± 17.1 μg kg^{-1} was obtained in VFX-enriched feed, whereas, VFX was not detected in CTR feed (Table 1). In seawater samples, detectable VFX levels were only found in VFX-water treatments (i.e. around 21 μg L^{-1} at days 14 and 28 and 16 μg L^{-1} at day 35; Table 1). As for non-contaminated fish samples (i.e. Control treatment), despite VFX was not detected in seawater samples nor in CTR feed, liver and brain of control fish samples exhibited low, but still quantifiable levels of VFX (i.e. around 22 μg kg^{-1} in the liver at days 28 and 35, and around 2 μg kg^{-1} in the brain only at day 28). Thus, these values were subsequently accounted for when analysing data obtained in contaminated fish.

3.2. VFX uptake and elimination via water

VFX concentration in tissues of fish exposed via contaminated seawater, as well as the tissue:plasma concentration ratios are shown in Fig. 2 and Table A.3. VFX concentration significantly varied according to tissue, with liver being the primary organ for VFX uptake, regardless of seawater temperature and pCO_2 conditions (6786.4 ± 1176.7 μg kg^{-1} dw, equivalent to an average NAR of 241.6 μg kg^{-1} day^{-1} in VFX-water treatment, and 3692.1 ± 272.9 μg kg^{-1} dw equivalent to an average NAR of 131.1 μg kg^{-1} day^{-1} in Acid+Warm+VFX-water treatment; $p < 0.05$; Figs. 2A and 3A). Furthermore, significantly higher liver:plasma concentration ratios were observed in fish exposed to the reference temperature a pCO_2 conditions (5.3 ± 1.2 and 9.8 ± 2.5 at days 28 and 35, respectively; Fig. 2C). On the other hand, significantly lower VFX concentrations were found in the other fish tissues, particularly, in the muscle (423.3 ± 159.2 μg kg^{-1} dw equivalent to an average NAR of 15.1 μg kg^{-1} day^{-1}, and 329.2 ± 44.8 μg kg^{-1} dw equivalent to an average NAR of 11.8 μg kg^{-1} day^{-1}, in treatments VFX-water and Acid+Warm+VFX-water, respectively; Figs. 2A and 3A). Furthermore, significant and negative correlations were found between morphometric data and VFX NARs in these three tissues (r ranging between −0.86 and −0.95; $p < 0.05$; Table 3).

Regarding the combined effects of abiotic variables, warming combined with acidification resulted in significantly higher TW and TL compared to those observed in fish exposed to the reference temperature and pCO_2 conditions ($p < 0.05$; Table 2), and such increase was accompanied by an overall reduction of VFX concentrations and tissue:plasma concentration ratios ($p < 0.05$; Fig. 2A and C), as well as significantly lower NARs and BCFs in plasma, brain and liver tissues ($p < 0.05$; Fig. 3A and B).

During the elimination phase (day 35), overall, increased temperature and pCO_2 facilitated VFX elimination in the liver and muscle tissues (i.e. EF maximum values up to 32% and 22%, respectively; $p < 0.05$; Fig. 3C). Yet, such trend was not observed in fish brain, as no elimination was observed in this tissue, regardless of seawater temperature and pCO_2 conditions (Figs. 2B and 3C).

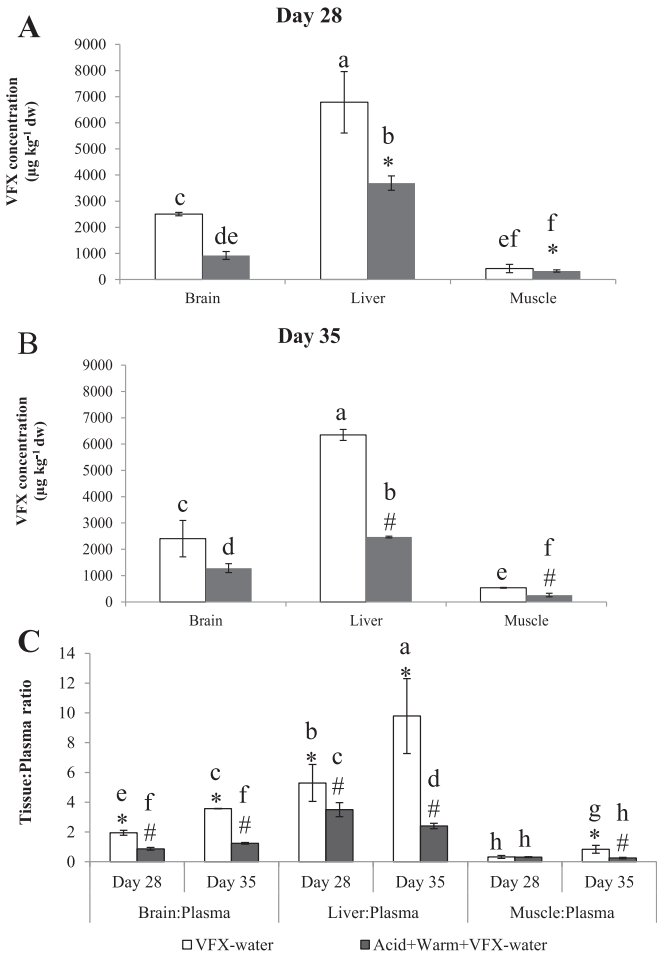

Fig. 2. Venlafaxine (VFX) concentrations (μg kg^{-1} dw) in tissues of fish exposed to VFX via water at day 28 (exposure phase; 2A) and day 35 (elimination phase; 2B), as well as, tissue to plasma VFX concentration ratio at days 28 and 35 (2C). Different letters indicate significant differences between tissues and treatments, whereas different symbols (* or #) indicate significant differences between day 28 and day 35 for the same tissue ($p < 0.05$). Abbreviations: Acid − simulated acidification (i.e. pCO_2 ~1500 μatm, equivalent to pH = 7.6 units); Warm − simulated warming (i.e. T = 24 °C).

3.3. VFX uptake and elimination via feed

VFX concentration in tissues of fish exposed via feed, as well as the tissue:plasma concentration ratios are shown in Fig. 4 and Table A.3. Despite values in VFX-feed treatments were much lower than those observed in VFX-water treatments (i.e. maximum VFX concentration = 189 μg kg^{-1} dw in liver; Figs. 2 and 3), tissues' VFX bioaccumulation followed a similar pattern, with the liver also being the primary organ for the bioaccumulation of this contaminant regardless of treatment (e.g. in VFX-feed treatment: 26.7 ± 86.5 μg kg^{-1} dw equivalent to an average NAR of 3.7 μg kg^{-1} day^{-1}; $p < 0.05$), followed by the brain, whereas much lower VFX concentrations were found in plasma and muscle (Figs. 4A and 5A). In addition, under the reference temperature and pCO_2 conditions, liver:plasma concentration ratio was also significantly higher than those observed in the brain and muscle at day 28 (10.7 ± 5.3; $p < 0.05$; Fig. 4C). As observed in VFX-water treatments, significant

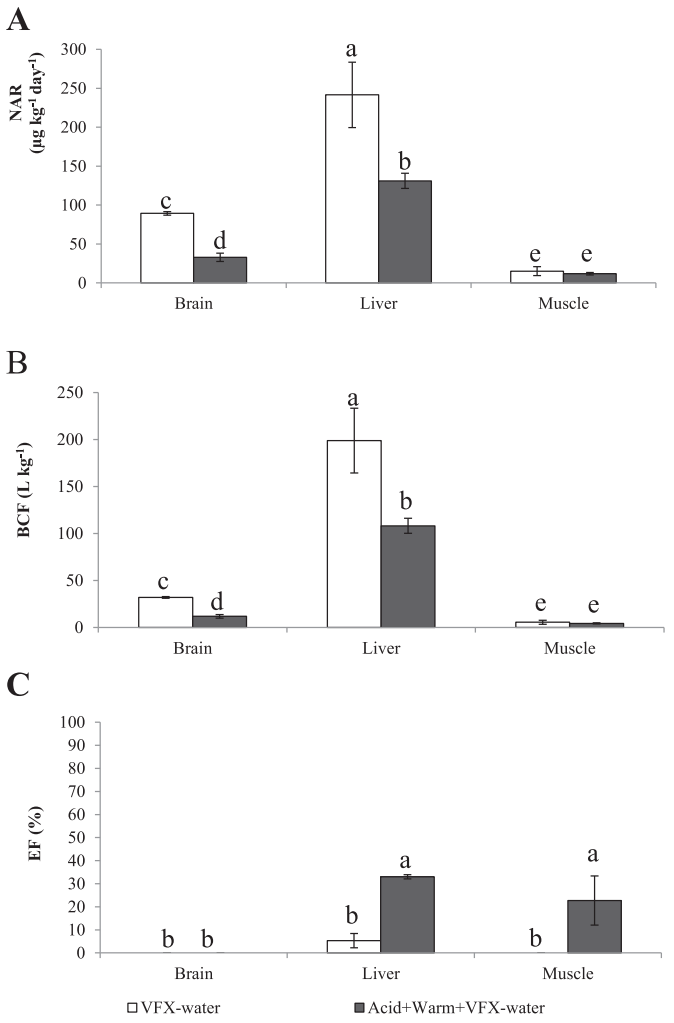

Fig. 3. Venlafaxine (VFX) net accumulation rates at day 28 (NAR; µg kg^{-1} day^{-1}; 3A), bioconcentration factors (BCF; L kg^{-1} ww; 3B) and VFX elimination factors at day 35 (EF; %; 3C) in VFX-water treatments. Different case letters indicate significant differences ($p < 0.05$). Abbreviations: Acid − simulated acidification (i.e. pCO_2 ~1500 µatm, equivalent to pH = 7.6 units); Warm − simulated warming (i.e. T = 24 °C).

and negative correlations were also found between biometric data and VFX NARs in fish plasma ($r = -0.67$ and -0.72 for TW and TL, respectively; $p < 0.05$), but not in the three fish tissues (Table 3).

As for the effects of seawater temperature and pCO_2, overall, warming (with or without the combination of acidification) promoted a significant increase in fish TW and TL compared to the reference temperature and pCO_2 conditions ($p < 0.05$; Table 2). In parallel, similar to what was observed in VFX-water treatments, increased temperature and pCO_2 levels, acting alone or in combination, resulted in significantly lower VFX concentrations and NARs in the liver at day 28 (i.e. between 55.8 and 78.2 µg kg^{-1} dw, equivalent to average NARs between 1.7 and 2.0 µg kg^{-1} day^{-1} in Acid+VFX-feed and Warm+VFX-feed treatments; $p < 0.05$; Figs. 4A and 5A). Conversely, significantly higher VFX bioaccumulation was also observed in fish brain when warming acted alone (80.5 ± 2.0 µg kg^{-1} dw, equivalent to a NAR of 2.8 µg kg^{-1} day^{-1} in treatment Warm+VFX-feed; $p < 0.05$), but not when acidification was combined (i.e. Acid+Warm+VFX-feed; Figs. 4A and 5A). Furthermore, the lower VFX tissue concentrations along with enhanced VFX plasma concentrations under warming and acidification (see also Appendices, Table A.4), overall, decreased the tissue to plasma concentration ratios, particularly when both stressors were combined (Fig. 4C).

After the 7 days of elimination phase, distinct patterns were observed according to fish tissue and treatment (Figs. 4B and 5B). Fish exposed to warming and acidification exhibited lower VFX elimination in the liver, particularly when both abiotic stressors were combined (9.1 ± 3.0% in treatment Acid+Warm+VFX-feed) compared to those exposed to the reference conditions ($p < 0.05$; Fig. 5B). As for the brain and muscle, no significant differences were observed between treatments simulating the reference and increased seawater temperatures (i.e. between VFX-feed and Warm+VFX-feed and Acid+Warm+VFX-feed; $p > 0.05$), with EF values reaching up to 45.6% in the brain, and up to 100% in the muscle (Figs. 4B and 5B). Conversely, acidification alone (i.e. Acid+VFX-feed) resulted either in very low VFX elimination (14.7 ± 4.4% in the liver), or in no elimination at all (in the remaining tissues; $p < 0.05$; Figs. 4B and 5B). Regarding tissue:-plasma concentration ratios, significant differences between days 28 and day 35 were only found in liver of Acid+VFX-feed and Acid+Warm+VFX-feed treatments, as well as in muscle of VFX-feed treatment ($p < 0.05$; Fig. 4C).

4. Discussion

4.1. VFX levels in feed, seawater and non-contaminated fish samples

VFX was not detected in the CTR feed, indicating that no external contamination occurred during feed preparation. Furthermore, these results assured that seawater was the only route of VFX exposure in VFX-water treatments during the first 28 days of trial, as well as that VFX dietary exposure did not occur during the elimination phase in all treatments (see Table 1).

As for seawater samples, the results obtained evidenced that: i) no other source of contamination occurred in VFX-feed treatments, apart from the intended dietary VFX exposure; ii) VFX concentrations were steady in VFX-water treatments throughout the 28 days of exposure phase; and iii) the 25% of daily water renewal carried out in each incubating tank during the last 7 days of trial (i.e. elimination phase) diminished VFX concentrations in seawater, but

Table 3
Pearson correlation coefficients between biometric data and venlafaxine (VFX) net accumulation rate (NAR). In each column, asterisks indicate significant correlations. Abbreviations: TW − total weight; TL − total length; VFX-water − fish exposed to VFX via seawater; VFX-feed − fish exposed to VFX via feed.

		TW vs TL	NAR vs TW	NAR vs TL
VFX-water treatments	Plasma	**0.98***	**−0.86***	**−0.92***
	Brain		**−0.91***	**−0.95***
	Liver		**−0.87***	**−0.90***
	Muscle		−0.57	−0.63
VFX-feed treatments	Plasma	**0.97***	**−0.67***	**−0.72***
	Brain		0.31	0.27
	Liver		−0.40	−0.41
	Muscle		0.01	0.00

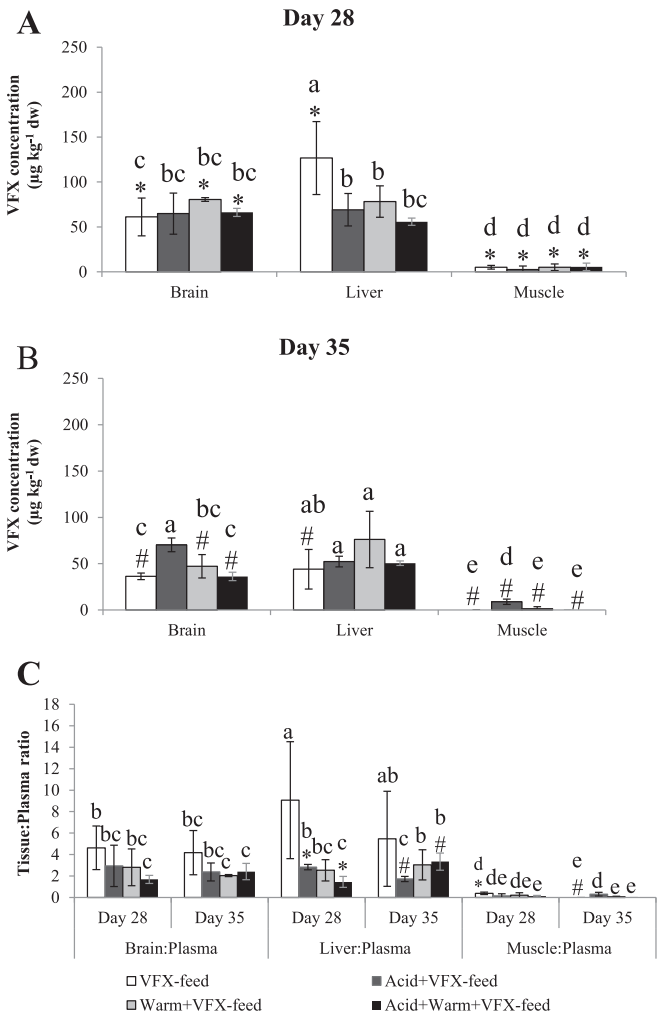

Fig. 4. Venlafaxine (VFX) concentrations (µg kg^{-1} dw) in tissues of fish exposed to VFX via feed at day 28 (exposure phase; 4A) and day 35 (elimination phase; 4B), as well as, tissue to plasma VFX concentration ratio at days 28 and 35 (4C). Different letters indicate significant differences between tissues and treatments, whereas different symbols (* or #) indicate significant differences between day 28 and day 35 for the same tissue ($p < 0.05$). Abbreviations: Acid – simulated acidification (i.e. pCO_2 ~1500 µatm, equivalent to pH = 7.6 units); Warm – simulated warming (i.e. T = 24 °C).

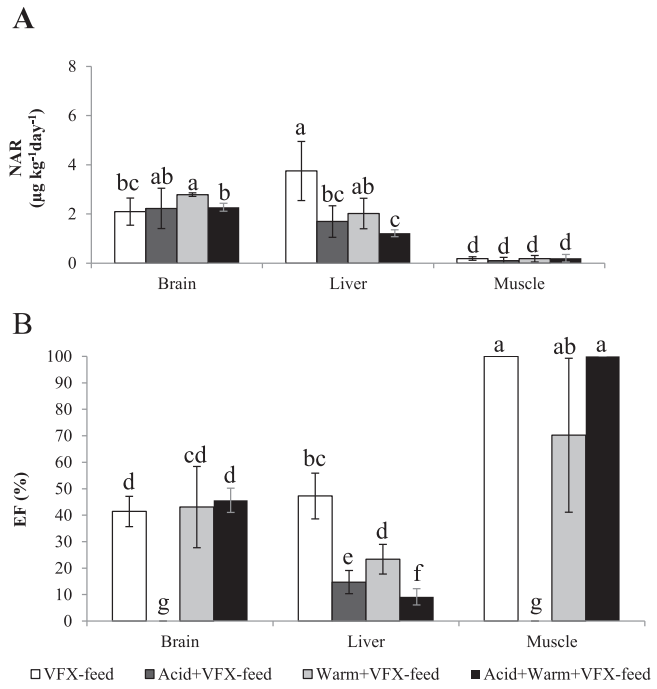

Fig. 5. Venlafaxine (VFX) net accumulation rates at day 28 (NAR; µg kg^{-1} day^{-1}; 5A) and VFX elimination factors at day 35 (EF; %; 5B) in VFX-feed treatments. Different letters indicate significant differences among VFX-feed treatments ($p < 0.05$). Abbreviations: Acid – simulated acidification (i.e. pCO_2 ~1500 µatm, equivalent to pH = 7.6 units); Warm – simulated warming (i.e. T = 24 °C).

did not enable a complete removal of this compound in VFX-water treatment. Furthermore, it should also be noted that VFX levels found in seawater samples from VFX-water treatments at day 35, might also be partially due to the potential desorption of VFX from fish skin and tank glass walls during the clearance phase (previously adsorbed during the exposure phase). Finally, VFX was not detected in CTR feed nor in seawater samples from the Control treatment, but was still quantified at low levels in brain and liver composite samples of non-contaminated fish samples (Table 1), suggesting that an external contamination might have occurred at some point, most likely during sample collection, processing and/or analysis due to contaminated materials (e.g. sample recipients, tools) and/or equipment.

4.2. VFX exposure route, tissue distribution, uptake and elimination

To the best of author's knowledge, there are no studies available concerning other VFX exposure pathways apart from water (e.g. Brooks, 2014; Zenker et al., 2014). Nevertheless, uptake mechanisms apart from contaminant passive diffusion across the gills (i.e. inhalation) can be particularly important in the case of PPCPs: these compounds are highly ionisable and cover a wide range of molecular polarity and, therefore, other specific biochemical mechanisms (e.g. ion trapping, protein binding) should also be accounted in terms of compound uptake and bioavailability (Klosterhaus et al., 2013; Stott et al., 2015). Furthermore, dietary exposure can have a preponderant role on contaminants' bioaccumulation, especially in marine predatory species with long life cycles, such as *A. regius* (Dijkstra et al., 2013; Brooks, 2014; Zenker et al., 2014; Maulvault et al., 2016).

The two exposure routes led to different VFX tissue concentrations and NAR; i.e. NAR in VFX-water treatments was up to 50 times higher than in VFX-feed treatments (see Figs. 2–5). This discrepancy can be attributed to the different VFX concentrations that were selected for water and feed exposure and, as previously mentioned, such selection was based on: i) VFX exposure via feed: VFX levels commonly found in species inhabiting contaminated coastal areas, susceptible to accumulate this contaminant, and that are natural preys of juvenile meagre (Martínez Bueno et al., 2014; Álvarez-Muñoz et al., 2015), as well as the VFX exposure levels which were expected to elicit the bioaccumulation of this compound to detectable levels during the timeline of the trials; and ii) VFX exposure via water: the lowest VFX concentration range previously reported to cause significant behavioural effects following short-term VFX exposure (Bisesi et al., 2014). Thus, it should be noted that the present results should be looked at as a proof of concept, since the exposure water concentration is above values usually found in water bodies, and that VFX uptake patterns may depend on exposure dose and time (Brooks, 2014; Zenker et al., 2014; Silva et al., 2016). Furthermore, the present experimental design did not allow to investigate other parameters (e.g. respiration and ingestion rates, compound bioavailability), which would be crucial to draw conclusions regarding the predominance of one

exposure route over the other. Yet, a recent multi-trophic marine study, reported that dietary exposure did not have a preponderant role compared to other exposure pathways, on the uptake of the antidepressants sertraline and fluoxetine (also weak basic pharmaceuticals) (Boström et al., 2017).

When it comes to contaminant uptake, the favouring of one exposure route over another can be closely linked to the physical-chemical properties of the compound, particularly to its octanol-water partition coefficient (log Kow; Qiao et al., 2000). In general, compounds that have a log Kow <3 have been pointed out to be mostly uptaken through inhalation, whereas those with log Kow >6 are mostly uptaken through ingestion (e.g. Heath, 1995; Qiao et al., 2000). However, for ionisable compounds like VFX, log Dow instead of log Kow should be considered in order to account for the ionization of the compound in its partition. Venlafaxine has a log Dow of 1.78 at pH 8, which is lower than its log Kow (2.74–3.30; Aryal et al., 2012), suggesting that this compound can rather be uptaken by inhalation than ingestion. Nevertheless, this parameter cannot be used alone to explain the differences observed in the bioaccumulation of the different exposure routes since other physical-chemical properties can affect the uptake (Huerta et al., 2016a,b). Thus, the present results also point out that VFX has the potential to be biomagnified by fish, i.e. to be transferred from food to biota (i.e. dietary exposure through the ingestion of a contaminated prey or a part of it), and such biomagnifying potential, along with biochemical and behavioural alterations in marine biota after chronic exposure to antidepressants (e.g. Valenti et al., 2012; Bisesi et al., 2014; Maulvault et al., 2018a), emphasize the ecological hazards they can pose to marine ecosystem.

Concerning VFX tissue partitioning, results showed that, regardless of exposure pathway, liver was the primary tissue for the uptake of VFX (see Figs. 2–5); this organ is responsible for the metabolization and redistribution of xenobiotics to other tissues, through the blood circulation (Wang et al., 2013; Maulvault et al., 2016). A similar pattern of compound partitioning in fish tissues and plasma (see Figs. 2–5, as well as Appendices, Table A.4) was also reported in previous studies focusing on VFX exposure in fish species (i.e. VFX concentration: liver > brain > muscle, in brook trout, *Salvelinus fontinalis*; Lajeunesse et al., 2011; VFX concentrations: liver > brain > plasma > muscle, in rainbow trout, *Oncorhynchus mykiss*; Grabicova et al., 2014), as well as on other common antidepressants (citalopram, sertraline and fluoxetine: liver > brain > plasma and muscle, in rainbow trout; Grabicova et al., 2014). In general, higher BCF values were observed in the present study (water exposure; Fig. 3B) compared to those previously reported (Lajeunesse et al., 2011; Grabicova et al., 2014). For instance, in the recent study of Grabicova et al. (2014) performed on juvenile rainbow trout, the BCF values obtained after 13 days of exposure to VFX (330 ng L^{-1}) were below 6 L kg^{-1} in fish muscle, and up to 63 L kg^{-1} in liver. Similarly, lower VFX BCFs (between 2 and 18 L kg^{-1}) were also calculated in brook trout exposed to municipal wastewaters (VFX concentration ~40 ng L^{-1}) for a period of 3 months (Lajeunesse et al., 2011). Yet, the BCF obtained following 28 days of water exposure suggest that VFX is not bioaccumulative in fish tissues, based on the thresholds set by regulatory authorities (i.e. BCF >1000 L kg^{-1} ww; EPA, 2012; or BCF >2000 L kg^{-1} ww; EU, 2011), and such results are consistent with previous findings (e.g. Lajeunesse et al., 2011; Grabicova et al., 2014; Serra-Compte et al., 2018). On the other hand, considering the different experimental conditions among studies, yielding discrepant BCF values, it can be hypothesized that the uptake mechanisms of antidepressants, such as VFX, may not only differ according to species (including possible differences between fish sizes, gender and other ecological features), but also be dependent on the exposure dose and time (e.g. Brooks, 2014; Zenker et al., 2014; Silva et al., 2016). Given the current lack of empirical data on this matter, further research should be carried out particularly considering different exposure concentrations and times, in order to better understand the toxicokinetics of these pharmaceuticals in the marine environment.

When assessing the bioconcentration/bioaccumulation of chemical contaminants, animal size and/or lipid content are two aspects that should also be considered, as these variables are often correlated with contaminants' tissue concentrations (Dijkstra et al., 2013; Zenker et al., 2014; Maulvault et al., 2016). Indeed, the present results revealed that, after 28 days of VFX exposure via water, VFX bioconcentration was strongly (and negatively) correlated to animal size (except in fish muscle; see Table 3), indicating that VFX concentration, in general, tends to decrease in fish tissues and plasma as the animal size increases, most likely due to somatic growth dilution (Dijkstra et al., 2013). On the other hand, the absence of a significant correlation between fish size and tissue bioaccumulation in VFX-feed treatments (except in plasma) may be related to the exposure dose (i.e. the final concentration in VFX-enriched feed), as well as to the distinct bioavailability mechanisms of this compound according to the exposure route (Sanchez and Meier, 1997; Brooks, 2014; Gray and Hughes, 2015).

Finally, regarding the elimination phase, the present results showed a very low persistence of VFX in the muscle of fish exposed via feed, partially due to the much lower bioaccumulation found in this tissue (see Figs. 4 and 5). Furthermore, low persistence was also observed in the liver of VFX-feed fish, which is in agreement with the following facts that liver is a primary organ for contaminant metabolization and its subsequent detoxification (Aryal et al., 2012; Maulvault et al., 2016, 2017). In contrast, no elimination was observed in fish tissues exposed to VFX via water under the reference temperature and pCO_2 conditions (see Figs. 2 and 3). Such results were most likely due to the high tissue concentrations reached in this treatment (in line with high water exposure concentration), as well as the presence of VFX in seawater during the elimination phase of the trial.

4.3. *Effects of warming and/or acidification and the link with seafood safety*

Despite the limited information about emerging contaminants in terms of ecotoxicology and food safety, recent studies suggest that abiotic variables (e.g. changes in temperature, pH, light and dissolved oxygen) can play a key role on PPCPs' chemical behaviour, degradation, metabolization and toxicity (Farré et al., 2008; Burke et al., 2014; Qian et al., 2015).

In general, marine organisms subjected to warmer temperatures exhibit enhanced metabolism, accompanied by increased ventilation and feeding rates in response to higher metabolic demands. Such changes often translate into a higher uptake of contaminants dissolved in the water column (via respiration) or present in feeds or natural preys (via ingestion; e.g. Schiedek et al., 2007; Manciocco et al., 2014; Maulvault et al., 2016; Sampaio et al., 2016). For instance, a recent study showed that sotalol and sulfamethoxazole bioconcentration on *Mytillus galloprovincialis* did increase with water warming, but VFX bioconcentration actually decreased in the same warming conditions (Serra-Compte et al., 2018). Contrasting this trend, in the present work, warming generally resulted in lower VFX tissue uptake (regardless of exposure route), with the exception of fish brain from Warm+VFX-feed treatment (see Figs. 2–5). Such decrease could be due to the fact that enhanced metabolic rates at higher temperature can also promote increased contaminant elimination rates, i.e. contaminant metabolization and subsequent excretion, thus translating in lower contaminant bioaccumulation in fish tissues (Schiedek et al., 2007; Manciocco et al.,

2014; Maulvault et al., 2016; Sampaio et al., 2016). Noteworthy, the lower VFX uptake at higher temperatures (i.e. in the liver of VFX-feed and in brain and liver of VFX-water treatments) could also be related to the somatic growth dilution, given the higher TW and TL (i.e. enhanced growth rates; see Table 2) observed in treatments simulating seawater warming (e.g. Dijkstra et al., 2013; Maulvault et al., 2016; Anacleto et al., 2018).

Concerning VFX elimination, warming (alone or combined with acidification) led to diminished VFX EF in the liver (a primary organ for metabolic breakdown of xenobiotics and subsequent transport to other organs and/or excretion) compared to the reference temperature conditions (see Fig. 5B), suggesting that the physiological stress induced by +5 °C of temperature increase not only affected the excretion of VFX in its parental form, but also compromised its transformation into different metabolites at the liver. In agreement with this, in a previous study on juvenile *Dicenthrachus labrax* exposed to MeHg, liver's ability to detoxify this pollutant was also compromised under +4 °C of temperature increase (Maulvault et al., 2016). Thus, the physiological and metabolic alterations elicited warming, which resulted in lower elimination of VFX (parent compound) in the liver, also reinforced the need to consider, in future studies, the effects of the surrounding abiotic conditions when assessing compound metabolization mechanisms.

Seawater acidification has also the potential to directly or indirectly influence marine species' physiology and metabolism, therefore, affecting the way biota cope with the presence of contaminants (Freitas et al., 2016; Sampaio et al., 2018). Since most PPCPs are highly ionisable, but cell membranes are usually very poorly permeable to charged (ionic) contaminant forms, variations of the surrounding seawater pCO_2 levels are likely to influence the uptake and toxicity of this group of chemical contaminants (e.g. Orvos et al., 2002; Rowett et al., 2016; Serra-Compte et al., 2018). However, in this study VFX was mostly present positively charged under both pH 8.0 units and pH 7.6 units (VFX dissociation constant (pKa) = 9.6; Singh et al., 2008) and, therefore, physical-chemical properties of the compound would not be affected by pH. An increment in VFX bioaccumulation in brain and plasma (see Figs. 4 and 5 and Table A.3) was observed under lower pH levels (Acid+VFX-feed) compared to the reference pH conditions regardless of temperature (VFX-feed and Warm+VFX-feed), although a decrease in liver and muscle bioaccumulation was also observed. Thus, physiological alterations induced in fish exposed to this treatment may explain the observed variations (e.g. Rosa et al., 2016; Sampaio et al., 2016, 2018), as well as the possible damages elicited by hypercapnia in apical epithelial membranes of fish tissues, which can then facilitate contaminant penetration into cells (Freitas et al., 2016; Sampaio et al., 2016, 2018; Shi et al., 2016; Velez et al., 2016).

On the other hand, the altered physiological condition and metabolic activity promoted by lower pH levels may also justify the significantly lower VFX elimination in liver compared to reference conditions. Contrasting the present findings, Serra-Compte et al. (2018) observed an increment in VFX elimination in mussels under acidification conditions.

As for the combination of warming and acidification, results showed that one abiotic variable may: i) enhance the effects of the other, i.e. VFX elimination in liver further decreased in Warm+Acid+VFX-feed fish, in relation to Acid+VFX-feed and Warm+VFX-feed; or ii) reverse the effects of the other, i.e. higher VFX bioaccumulation was observed in the brain of fish exposed via feed (Warm+VFX-feed), yet such increase at higher temperatures was counteracted by acidification (i.e. in Warm+Acid+VFX-feed). A recent study performed on marine bivalves exposed to a broad range of emerging contaminants also reported increased bioaccumulation of dechlorane 602 in treatments simulating warming combined with acidification, compared to treatments simulating a single stressor (Maulvault et al., 2018b). In contrast, this study further revealed that the effects of warming and acidification acting alone on TBBPA and PFOS bioaccumulation were reversed when these stressors were combined. Following this trend, Sampaio et al. (2018) reported that reduced pH also countered MeHg increased bioaccumulation observed in juvenile meagre *A. regius* exposed to warming in isolation. In VFX water exposed treatments an opposite trend was observed in fish liver, where VFX elimination in Warm+Acid+VFX-water increased when compared to the reference temperature and pCO_2 conditions (Figs. 3 and 5). This difference suggests that: i) exposure route may have a stronger impact in VFX bioaccumulation and elimination mechanisms than the surrounding abiotic conditions; and ii) the different bioaccumulation and elimination patterns observed according to the exposure route may actually be dose dependent.

Finally, VFX has been detected in commercially important seafood species together with other PPCPs, although at relatively low levels (e.g. up to 36.1 ng g^{-1} dw in bivalve species; Álvarez-Muñoz et al., 2015; up to 16.4 ng g^{-1} dw in brown trout, *Salmo trutta* m. *fario* Grabicova et al., 2017). This raises several concerns from the human health perspective, given the potential hazards to seafood consumers when ingesting contaminated species. Thus, there is a reinforcement that these pollutants should not only be regulated in seafood species, but also be included in regular environmental monitoring campaigns. In addition, the risks of PPCP's human exposure should be estimated considering both the present climate conditions and those foreseen for the ocean of tomorrow. Even though the results do not point out to increased VFX uptake in fish tissues in a climate change context, the trends observed in this study confirmed that seawater abiotic variables can have a strong influence on PPCPs' bioaccumulation and toxicity by impairing biota's mechanisms of contaminant bioaccumulation, metabolization and elimination. Such findings highlight that careful attention should be given to the expected effects of climate change when establishing maximum permissible limits for emerging environmental contaminants, such as PPCPs, in seafood and when defining human exposure recommendations to this group of contaminants.

5. Conclusions

Overall, data confirmed that VFX can be uptaken by fish species through contaminated water and diet. Seawater temperature and pCO_2 levels can strongly affect VFX uptake and elimination in marine organisms, by affecting contaminant physical and chemical properties, as well as animal metabolism and physiological responses. In general, warming resulted in increased VFX uptake in brain (and plasma), most likely due to the enhanced organisms' metabolic rates. In parallel, the diminished VFX uptake in liver might be attributed to enhanced VFX elimination rates at higher temperatures. In contrast, acidification resulted not only in lower VFX uptake in the liver compared to the reference pH conditions, but also in diminished elimination in all fish tissues, most likely due to the physiological stress induced in fish leading to impaired metabolic rates. The results also showed that a stressor may enhance the effects of another (e.g. VFX elimination in fish liver was further decreased when warming and acidification acted together), as well as reverse it (i.e. the higher VFX uptake in fish brain under warming alone was counteracted by acidification). The distinct patterns observed in the different scenarios strengthened the need to carry out greater research efforts to understand how multiple environmental stressors in a climate change context, such as warming, acidification and pollution, interact with each other, as well as how to integrate these variables in future regulations and recommendations regarding both the ecological and human health

impact.

Ethical statement

Fish trials were conducted according to legal regulations (EU Directive 2010/63), and approved by the Ethical Committee of the Faculty of Sciences of the Lisbon University, overseen by the Portuguese National Competent Authority (Direção-Geral de Alimentação e Veterinária, DGAV). All researchers and technicians involved in the maintenance, handling and sampling of live animals were certified in Laboratory Animal Sciences, by the Federation of European Laboratory Animal Science Associations (FELASA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.chemosphere.2018.06.004.

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